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Further Evidence on the Homogeneity of the Resistance to the Implantation of Malignant New Growths.

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Further Evidence on the Homogeneity of the Resistance to the Implantation of Malignant New Growths.

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This paper is based mainly on a study of the processes at the site of implantation of a malignant new growth when a secondary implantation is practised on mice already bearing transplanted tumours. New evidence will be adduced supporting the view that concomitantly with the establishment of such tumours, an active resistance may be induced by the absorption of tumour tissue. When a secondary inoculation fails, this failure is due to an active resistance to the cancer cells introduced, similar to that induced in normal animals by the absorption of tumour tissue or normal tissue of the same species. The process consists essentially in the cancer cells failing to elicit the specific connective tissue and vascular scaffolding necessary to their establishing themselves and growing into a tumour. In order to simplify still further prevailing conceptions of the process of immunity to cancer, we shall record corresponding observations on rats where the primary inoculation of a mouse carcinoma has been followed by a secondary inoculation of mouse tumour.

In the First Scientific Report of the Imperial Cancer Research Fund (March, 1904) it was stated "in the light of the phenomena of immunity, it is interesting to note that it is possible to obtain multiple tumours from trans-

plantations performed on the same date, and that transplantation can be successfully performed in animals in which tumours have already developed 14 days to 10 weeks after the first effective transplantation, *i.e.*, both when the primary tumour is small and when it has attained a large size." These positive results appeared to be important, first, because of their correspondence with the dissemination and formation of metastases in the normal course of the progress of cancer in man, and, secondly, because they afforded a basis for studying the conditions, favourable and unfavourable, to the establishment of secondary implantations in animals already bearing tumours, and hence had also an indirect bearing on the control of natural metastases. Therefore, these observations formed one of the starting points of our studies on the induction of resistance to the inoculation and growth of cancer. Their importance was enhanced later by the success attending efforts to reproduce experimentally both the local infiltrative and the disseminated lesions of the disease.

Other investigators, who, at a later date, were not so successful, either in re-inoculating animals already bearing tumours, or in reproducing the lesions of dissemination, have quite naturally drawn conclusions opposed to those drawn by us; for example, in April, 1906, Ehrlich reported "If metastasis formation be imitated experimentally by re-inoculating animals 8 to 10 days after they had been successfully inoculated with a rapidly growing tumour, then the second inoculation, whether it be made with the same or with a different tumour, does not take with few exceptions" (1). Ehrlich was led to attach enhanced importance to his observations by the fact that metastases were rare, or, if present, only of microscopical size, in his inoculated animals. He sought an explanation common to the two groups of observations in the assumption of "atreptic" immunity, meaning thereby that the rapidly growing tumour prevented successful re-inoculation and the establishment of metastases by withdrawing special nutritive substances (Substance X) from the circulation. The idea of "atreptic" immunity has been extended to explain also the transitory growth of mouse tumours in rats by assuming that growth ceased when the hypothetical Substance X, introduced with the graft, was exhausted. Ehrlich (2) modified his standpoint in 1908 in so far as to admit that re-inoculation may be successful although he maintains that the secondary tumours remain smaller than the corresponding tumours in control animals, and therefore he adheres to his assumption of the existence of an "atreptic" immunity.

The foregoing summary shows that the elucidation of the nature of active resistance to cancer has been complicated both by contradictory observations and by conflicting explanations of facts, regarding which there is complete

agreement. These difficulties are associated more especially with the results following upon the secondary inoculation of animals already bearing tumours as the result of primary inoculation. Some authors have succeeded where others have failed to obtain secondary tumours in this way. The methods by which secondary inoculations can be successfully carried out, the importance of dosage,* and other technical details, have been fully explained in a series of papers, and the contradictory results harmonised (3, 4, 5). It is however, necessary to meet hypothetical explanations of the reasons why a secondary inoculation may fail, by recording the results of actual observation of the processes responsible and comparing them with those following the induction of active resistance in normal animals. Therefore, the histological details of the process have been ascertained by examining the site of re-inoculation at definite intervals. This method is conveniently called the examination of "early stages."† By its means the true nature of the transplantation of cancer was demonstrated (6, 7), and later it revealed how important for the implanted cancer-cells was the provision of a specific supporting and nutritive scaffolding by the tissues of the new host (8). It also demonstrated, that in mice rendered resistant to carcinomata, there was a failure to supply this specific scaffolding, and hence the conclusion was arrived at that actively resistant animals robbed the cancer-cells of the chemiotactic powers they exercise on the connective tissues of the host (4). This failure of the specific stroma reaction was seen both when resistance was induced by the previous absorption of tumour tissue, and by the previous absorption of normal tissue—whether adult or embryonic—of the same species. For the elucidation of the mechanism of resistance there is, at present, no alternative to actual observation of the site of the inoculation of cancer-cells in living animals. Without resort to this laborious method the hypothetical assumption of an atreptic immunity was advanced to explain why a secondary inoculation may fail in an animal already bearing a tumour. By employing it the facts recorded in this paper have been elicited; they harmonise with what has been stated elsewhere on the successful re-inoculation of animals already bearing tumours, and show that the resistance which may exist to re-inoculation under these circumstances is identical in its nature and mechanism with that which can be induced in normal animals, *i.e.*, in the absence of a tumour.

It is immaterial which of the 65 tumour-strains growing in the laboratory

* It is impossible to compare the rate of growth of the tumours following inoculation if the amount of material inoculated as the starting point of growth is not stated. This factor still continues to be neglected, even in the most recent investigations of tumour growth. *Cf.* Moreschi, 'Zeitschrift f. Immunitätsforschung,' 1909.

† Fully described in the Scientific Reports of the Imperial Cancer Research Fund.

is used to illustrate the facts, since they agree for all the strains that have been tested, whether slowly or rapidly growing. Some tumour-strains illustrate a larger number of the sum total phenomena than others. Thus, strain "199" is very suitable for studying the relations existing between a growing tumour and the host bearing it. This tumour, an adeno-carcinoma of the mamma, has now been propagated for 15 months, and gives from 70 to 100 per cent. of tumours when inoculated into a fresh batch of mice. The inoculated tumour-cells proliferate very rapidly, so that, when the strain is in a positive phase of growth, from a dose of 0·05 gramme, tumours weighing about 1·5 grammes may be obtained after 10 days, in all the mice inoculated. The subsequent behaviour of the individual tumours of the same series is very variable; about a third of them will continue to grow rapidly during the next two to three weeks; another third will show a very much slower speed of growth for two to four weeks, and then resume a speed of growth somewhat slower than the original speed of proliferation; the remaining tumours will be gradually absorbed and disappear after 3 to 5 weeks. A single series of daughter tumours of this strain "199" illustrates the extremes of behaviour presented by other tumours, viz., those growing progressively and rapidly in all animals inoculated, and those exhibiting transitory proliferation only, even when implantation is successful in 100 per cent. of the animals inoculated. The parts played respectively by active resistance induced concomitantly with the development of the tumours, and by the qualities of the tumour-cells themselves in determining the different behaviour of different tumours have already been fully discussed (4, 5).

Advantage may be taken of the behaviour of tumour "199" to obtain a concise survey of the nature of the resistance or immunity against the re-inoculation of cancer in animals already bearing tumours. Series of mice, usually 20 in number, bearing growths 10 days old of tumour "199" in the right axilla, have been re-inoculated in the left axilla with another tumour either of the same strain or of another strain. Briefly, the result has been that the re-inoculation has failed to give rise to a tumour except in the cases where the tumour primarily inoculated has continued to grow rapidly. Where the primarily inoculated tumours have slowed up in their speed of proliferation, or where they have begun to diminish in size, the result of the re-inoculation has been that no tumours developed. In other words, the better the growth of the primary inoculation, the more favourable are the chances for a tumour developing from the second inoculation. This result confirms those previously recorded from this laboratory (5). The possibility can be excluded of the degree of hindrance to secondary inoculation being in direct proportion to the rate of growth of the primary tumour.

Investigation has also been made of the details of the processes at the site of the re-inoculation in mice 10 to 12 days after the primary inoculation of tumour "199." It has been found that there is an inhibition of the stroma reaction in those mice whose primary tumours had showed a slowing up in their speed of growth, or had begun to diminish in size. A study of the actual mechanism of resistance to re-inoculation points unambiguously to its being due to the induction of an active resistance to the cancer cells identical with that described for normal animals (4). It is possible for a mouse bearing a growing tumour to be actively resistant to re-inoculation, a fact which has already for other reasons been attributed to concomitant immunisation arising from absorption of a portion of the already established tumour (9). The successful re-inoculation of mice bearing the most rapidly growing "199" tumours is easily understandable on this basis, as in this case the amount of absorption of tumour tissue is practically *nil*, and resistance has not been established. The assumption of a special "atreptic" immunity in the case of animals already bearing tumours, where the host is passive and the tumour active, by withdrawing food, to explain the failure in certain cases to re-inoculate an animal the bearer of a growing tumour, is superfluous.

An immunity developing when cancer of one species is inoculated into another species of animal has been assumed by some workers to be an immunity against cancer. Only the most rapidly growing of mouse tumours when inoculated into rats grow for six to eight days according to Ehrlich (10), quite as well as in mice, but after this date they become absorbed, and the animals are then actively immune. If removed at the height of their development from the rats and inoculated into mice or rats, Ehrlich states they grow in the former but do not grow in the latter. The temporary growth in rats is explained by Ehrlich as another instance of atreptic immunity due to the absence in the rat of an unknown specific substance necessary for the growth of mouse tumours. The transitory growth of mouse tumours in rats has been shown to be common to those of slow growth (4) as well as of rapid growth, including Jensen's carcinoma, for which the possibility was denied. In the third scientific report of the Imperial Cancer Research Fund, the nature of the tissue reaction was demonstrated when rats, inoculated 14 days previously with mouse tumour, were re-inoculated, *i.e.*, while they were actively immune against mouse tumours. Further, this immunity was shown to be of a different character from the immunity of mice against mouse tumours. When rats immunised with mouse tumours were inoculated with "early stages" of a mouse tumour, there took place an active destruction of the introduced tumour

graft. After two to three days there was scarcely a single cancer cell left, whilst the surrounding rat tissues were proliferating actively and invading the dying graft. Here one had to deal with an active immunity against a foreign protein, and not with an immunity against a cancer cell as such. In spite of the observations made and recorded at that time, von Dungern and Coca (11) have since compared a similar phenomenon with the immunity against cancer from which it is so totally different, and have built up an hypothesis of "allergetic" immunity against cancer. They performed their immunity experiments with an epidemically occurring fibrocellular growth of the hare, which they have succeeded in transferring through eight generations in a strange species, viz., in rabbits. They find that when a rabbit has been successfully inoculated with this growth, it is impossible to obtain success with a re-inoculation. The site of re-inoculation is frequently the seat of a marked œdema, and they have found by histological examination of the site two or more days later, that there is extensive reaction with production of large mononuclear cells which are specially abundant in the veins and capillaries. They regard this reaction as being evidence of a tissue immunity, and especially of the nature of a local hypersensibility, in that the tissues having been once exposed to the action of these foreign cells, they react more energetically to second inoculation of them, apparently as the rat tissues have been described to do to re-inoculation of mouse tumour. Von Dungern and Coca have not made the direct observation that, in transferring this hare tumour to rabbits, they have been effecting a transplantation. If we accept their view, based upon indirect observations, that the transference of this tumour is really a transplantation, then their immunity results are comparable to those obtained in rats when inoculated with mouse tumour, with this difference, that it has been possible to propagate the hare tumour through several generations of rabbits. In a recent publication, von Dungern and Hirschfeld (12) claim that this local allergetic reaction is of great importance in its bearings on immunity to cancer.

The following experiments show how very different is the nature of the immunity produced by inoculating cancer of a strange species from the resistance to cancer produced by the absorption of tumour or normal tissues of the same species. In these experiments the behaviour of tumour-strain "199" has been studied in the rat. When 0·2 c.c. of this tumour is inoculated into rats, tumours 2 to 3 cm. long by 0·5 to 0·75 cm. broad are obtained in from eight to ten days. Histological examination of these tumours at about the eighth or ninth day has shown them to be composed almost entirely of granulation tissue, which in the rat is mainly fibroblastic

with a small central canal containing the inoculated tumour tissue, for the most part as necrotic *débris*. If examined at an earlier date, fifth or sixth day, small islets of carcinomatous tissue are seen lying in a very cellular tissue, the whole having the appearance almost of a carcinoma-sarcomatodes. If the tumour be removed from the rat at this earlier stage, and inoculated back into mice, it gives rise to tumours, but these do not grow so well as before *passage* through the rat, and the percentage of tumours obtained is only about a third of the previous percentage.

Nine rats, weighing 490 grammes, were inoculated on the dorsum with 0·2 c.c. of an emulsion of mouse tumour "199." Three days later, other nine rats, weighing 510 grammes, were inoculated in exactly the same way with the same tumour; and again, three days later, a third batch of nine rats, weighing 540 grammes, were treated with the same tumour in the same manner. Three days after inoculation of the last batch, that is nine, six, and three days respectively, after the primary inoculation, these three batches of rats were tested simultaneously by inoculating "early stages" of another mouse tumour in the right axilla. Twelve normal rats, weighing 455 grammes, were also inoculated with early stages to serve as control. These early stages were removed at 24, 48, 72 hours after inoculation, and examined in serial sections. In the normal control rats the "early stages" continued to grow for the first three days. The tumour cells continued to divide, whilst the introduced stroma degenerated, and was beginning to be replaced by a fresh stroma from the rat tissues on the third day. In the batch of rats treated three days previously with 0·2 c.c. of mouse tumour, no difference could be seen in the early stages as compared with the control early stages in normal rats. There was no marked difference, also, in the case of the batch treated six days previous to inoculation of the early stages. In the batch of rats which had been treated nine days previous to testing with "early stages," quite a different state of affairs obtained. In the grafts examined after 24 hours' residence, a large number of the introduced tumour cells, both in the peripheral and central parts of the graft, had been killed. Further, there was a complete absence of mitoses of the tumour cells. Mitotic division of the adjacent connective tissue cells of the rat was already present, and there was a large exudate of polymorphonuclear leucocytes. At 48 hours only a few tumour cells, mostly in the central parts of the graft, had retained their morphological characters. By 72 hours, all the tumour cells had been killed off, and an abundant reaction tissue was being formed by the rat tissues.

Such experiments demonstrate in the clearest manner that after inoculation of a given dose of mouse tumour into a rat, there is produced between

the sixth and ninth day an active immunity which leads to the rapid destruction of any mouse tumour cells subsequently introduced. Further, it can be inferred that the production of this active immunity contributes to terminating the transitory growth of mouse cancer-cells in rats. The cessation of their growth is due largely to active immunity concomitantly induced, and to other influences directly injurious to them. The immunity induced in the rat against mouse cancer is very different from the active resistance of a mouse to inoculation of tumour of its own species, for, as demonstrated in 1908, mouse tumour cells can live and divide for eight to ten days in a resistant mouse, but they are incapable of eliciting the stroma reaction necessary for their establishment as a fresh tumour (4).

It has been possible to demonstrate the essential difference between these two types of immunity in another manner. Up to the present it has been found possible to render an animal resistant to tumours of its own species, only by previous inoculation of *living* tissues also of its own species. It is shown in another communication that if mouse tumour be disintegrated by pounding in a mortar cooled in salt and ice, and then be inoculated into mice, it does not give rise to the development of tumours, neither does it produce any immunity against mouse tumours; on the contrary, hypersensitiveness may result (13). Rats inoculated with a dose of 0·25 c.c. of mouse tumour ground up in this way have then been tested by introducing "early stages" of a mouse tumour. It has been found that disintegrated mouse tumour does render rats actively immune against mouse tumours. The fundamental distinction between the behaviour of disintegrated mouse tumour in mice and rats respectively, demonstrates that the immunity induced in the rats is directed not against mouse cancer *quâ* cancer, but against mouse cancer *quâ* mouse tissue, and it is exactly analogous to the anti-bodies obtained by inoculation of heterologous tissues, in the production of precipitins, hæmolysins, and cytotoxins. Indeed, corresponding reactions have been obtained *in vitro* by means of disintegrated tumour tissue inoculated into strange species (9); whereas the true reactions of induced resistance to cancer are only obtained *in vivo* by inoculation of living tumour, or tissue of the same species.

In confirmation of previous results, animals already bearing rapidly growing tumours can be re-inoculated successfully, and conclusions based upon experimental conditions permitting of the reproduction of the features of dissemination, are more worthy of consideration than conclusions based upon experimental conditions which exclude such reproduction.

The presence of an experimental tumour is compatible with the existence of active resistance to re-inoculation.

The assumption of an "atreptic" immunity to cancer has been applied to phenomena which are more naturally explained as due to an active resistance induced against cancer-cells. The assumption of a special form of immunity, "atreptic" in nature, is also incompatible with the fact that re-inoculation is successful in direct relation with the rapidity of the growth of the successful primary inoculations, and with the fact that when resistance to re-inoculation is present, it is identical with that active form of resistance induced in normal animals by the absorption of living tissue, either tumour or normal.

The assumption of an "allergetic" immunity to cancer is applied to what is really an active immunisation against the tissues of a strange species of animal.

The immunity induced in rats by the inoculation of mouse cancer, or by similar heterologous inoculations in the case of other animals, is absolutely distinct from the resistance induced against cancer of the same species to which it has no direct relation.

Only one form of active resistance to the implantation of cancer from one animal to another has as yet been demonstrated to exist. This resistance follows only upon the absorption of living tumour tissue or living normal tissue of one animal, when introduced into another animal of the same species. So far as yet elucidated, it consists primarily in an inhibition of the specific chemiotactic powers which cancer-cells exercise upon the connective and vascular tissues of the host. This single explanation harmonises all the observed facts, and should rid the experimental study of cancer both of confusion and error. It may be pointed out that investigations, such as those described in this paper, bear upon the nature and biological behaviour of cancer-cells; but that they do not admit of any inferences as to possible methods of treating the disease.

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